Positive and Negative Regulatory Elements in the HIV-1 5′UTR Control Specific Recognition by Gag

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Background

- Human Immunodeficiency Virus type 1 (HIV-1) is responsible for the development of Acquired Immunodeficiency Syndrome (AIDS).
- Approximately 37 million people are currently infected by the HIV-1 worldwide (WHO).
- A cure for HIV-1 has yet to be developed, but the disease can be managed through a combination of antiretroviral medications.
- However, the continuous use of antiretroviral medications drives the risk for the emergence of viral resistance mutations and drug failure. In turn, the demand for novel antiretroviral medications remains at the highest priority for HIV-1 sufferers.

World Map of HIV-1 Prevalence

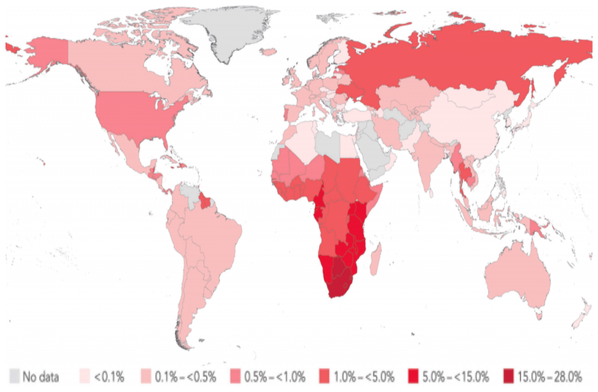


Figure from UNAIDS (2009)

HIV-1 Life Cycle

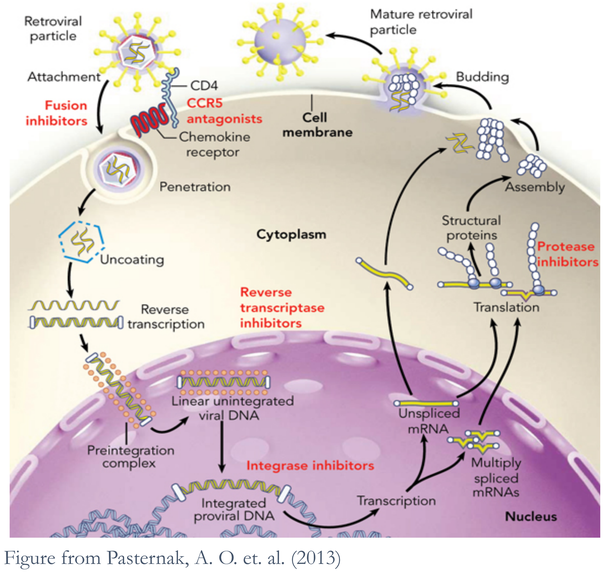


Figure from Pasternak, A. O. et. al. (2013)

Specific Interactions between Gag & Psi Facilitate Genomic RNA Packaging

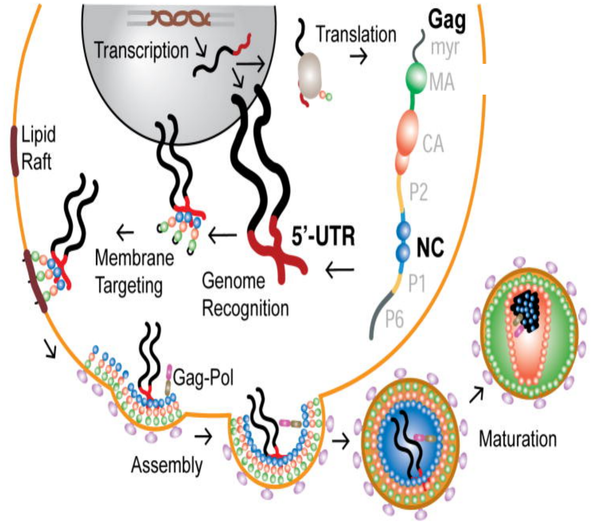


Figure from Lu, K. et. al. (2011)

Model: Psi-RNA Shifts Gag Binding Mode

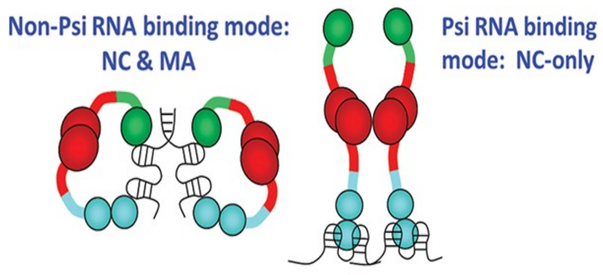


Figure from Webb, J. A. et. al. (2013)

Hypothesis: Positive and Negative Regulatory Elements Flank Psi in the 5′UTR

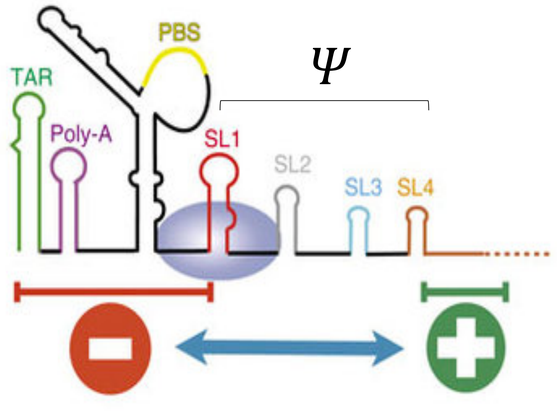


Figure from El-Wahab, E. W. et. al. (2014)

Experimental 5′UTR Constructs

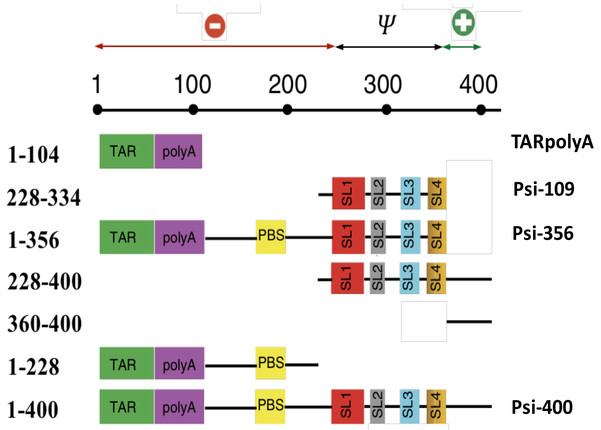
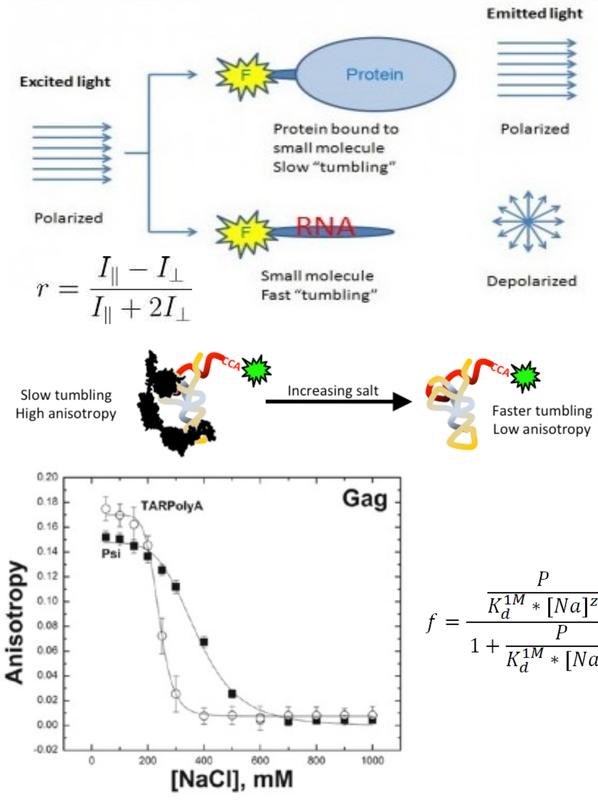


Figure adapted from El-Wahab, E. W. et. al. (2014)

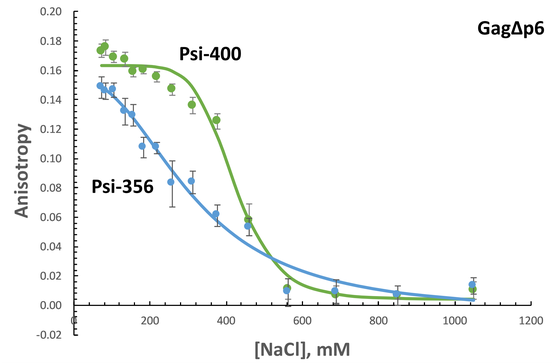
Fluorescence Anisotropy Based Salt Titration Assays



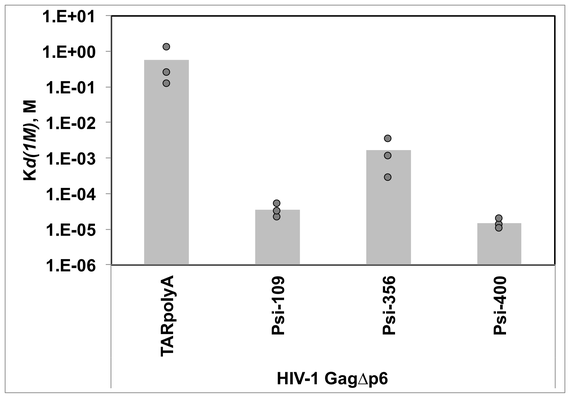
Figures from Webb, J. A. et. al. (2013)

- $K_{d(1M)}$: Describes the nonelectrostatic components of binding
- Z_{eff}: Reflects the number of charges medicating protein-RNA interaction

Positive Regulatory Element Required for Specific Gag Binding



GagΔp6		
RNA Variant (20.5 nM)	$K_{d(1M)}$	Z_{eff}
TARpolyA	5.6 (± 6.4) x 10^{-1}	10.5 (± 1.0)
Psi-109	3.6 (± 1.6) x 10^{-5}	5.4 (± 0.5)
Psi-356	1.6 (± 1.6) x 10^{-3}	8.1 (± 1.0)
Psi-400	1.5 (± 0.5) x 10^{-5}	2.3 (± 0.3)



- The data suggests that Psi-356 binds nonspecifically to GagΔp6 more like Non-Psi RNA, while Psi-400 binds specifically to GagΔp6 similar to Psi-RNA.
- This result is consistent with an upstream regulatory element required for specific Gag binding to the 5′UTR .

Future Work

- Complete salt titration assays on the other 5′UTR constructs.
- Run native gel assays to study RNA conformation.
- Explore the impact of RNA dimerization on Gag recognition by removing the dimerization initiation signal.

Acknowledgements

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